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Abeta-induced BACE-1 cleaves N-terminal sequence of mPGES-2.

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Editor

Biochemical and Biophysical Research Communications

RE:

"Abeta-induced BACE-1 cleaves N-terminal sequences of mPGES-2"

Dear Editor,

We would be very pleased if you would consider our manuscript for publication in ***Biochemical and Biophysical Research Communications***. Below is a brief summary of the background and purpose of our study.

In this report, we demonstrated that mPGES-2 can be cleaved by BACE-1, which leads to neuroinflammation in the brain. In addition to APP, novel substrates of BACE-1 have been identified. Furthermore, we demonstrated that amyloid beta treatment augmented protein levels of mPGES-2, which synthesizes PGE2. In addition, we revealed that a sequence of amino acids at the N-terminal of mPGES-2 is cleaved by BACE-1, which results in an activated form of mPGES-2. We also observed the translocation of mPGES-2, BACE-1, and COX-2 to the perinuclear space. Multiple lines of evidence, ranging from molecular and cellular to epidemiological data, have highlighted the importance of inflammation in the pathogenesis of Alzheimer's disease (AD). Our results imply that amyloid beta can evoke neuroinflammation by BACE-1-mediated cleavage of mPGES-2, which can aggravate the pathogenesis of AD.

We believe that this research is suitable for submission to ***Biochemical and Biophysical Research Communications***.

Sincerely,

Takeshi Kihara

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Title

Abeta-induced BACE-1 cleaves N-terminal sequence of mPGES-2

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Abstract

We previously indicated that amyloid beta (A β) augments protein levels of beta-site amyloid precursor protein cleaving enzyme-1 (BACE-1) through oxidative stress. In this study, we revealed that BACE-1 is involved in the cleavage of membrane-bound prostaglandin E2 synthase-2 (mPGES-2) in its N-terminal portion, which, in turn, enhanced the generation of prostaglandin E2 (PGE2). PGE2 results in increased A β production, initiating a cell-injuring cycle. Using rat primary cortical neurons, a 48 h treatment with A β 1-42 (5 μ M) resulted in the enhanced extracellular PGE2 levels up to about 1 ng/mL, which was attenuated by treatment with a BACE-1 inhibitor (200 nM). A synthetic peptide sequence of 20 amino acids that included the cleavage site of mPGES-2 (HTARWHL RAQDLHERS AAQLSLSS) was cleaved by recombinant BACE-1, confirmed using reverse-phase high-performance liquid chromatography. Cleaved or activated mPGES-2 augments the generation of PGE2. In addition, mPGES-2 was determined to be colocalized with BACE-1 and cyclooxygenase-2 in the perinuclear region in cells after exposure to A β . Exposure of neurons to PGE2 led to cell death, and A β production was enhanced by PGE2 (1 ng/mL, 48 h). Collectively, these results suggest that A β might cause neuroinflammation that aggravates Alzheimer's disease pathogenesis.

Keywords: A β ; Alzheimer's disease; BACE-1; COX-2; mPGES-2; PGE2.

Abbreviations used: A β , amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE-1, beta-site APP cleaving enzyme-1; COX, cyclooxygenase; EP, E prostanoid; cPGES, cytosolic prostaglandin E2 synthase-2; HPLC, high performance liquid chromatography; mPGES-1, microsomal prostaglandin E2 synthase-1; mPGES-2, microsomal prostaglandin E2

synthase-2; PGE2, prostaglandin E2; PGH2, prostaglandin H2; PGs, prostaglandins.

Introduction

It has been suggested that inflammation is involved in the pathogenesis of Alzheimer's disease (AD) [1]. Specifically, the accumulation of microglia around senile plaques and elevated levels of inflammatory cytokines, chemokines, proteases, and reactive oxygen species have been observed in the brains of AD patients. In and around the senile plaques and neurofibrillary tangles, microglial cells are activated, and the number of reactive astrocytes is increased. Collectively, these data have led to the hypothesis that brain inflammation is a cause of neuronal injury in AD.

Prostaglandins (PGs) are chemical mediators and potent inducers of inflammation. Cyclooxygenases (COXs) catalyze the conversion of free arachidonic acid to the endoperoxide intermediate PGG₂, which is then reduced by peroxidase activity to PGH₂. PGH₂ is metabolized rapidly, resulting in different end products depending on the relative abundance of synthases in different cell types. Examples of these end products include PGI₂, thromboxane A₂, PGD₂, PGE₂, and PGF₂ α. PGE₂ is also catalytically converted from PGH₂ by prostaglandin E synthase (PGES). It has been reported that levels of PGE₂ are increased in the cerebrospinal fluid (CSF) of AD [2], and the highest levels are observed in the very early stages of AD. These levels then decline with progressive cognitive impairment [3]. In vitro experiments have revealed that PGE₂ release is enhanced by amyloid beta (Aβ) [4], a major constituent of senile plaques found in AD brains. Furthermore, Aβ induced COX-2 activity may subsequently catalyze the production of PGE₂ [4].

The four receptor subtypes of PGE₂ are E prostanoid (EP) receptors 1-4. Deletion of the EP2 receptor in aged amyloid precursor protein (APP) Swe-PS1ΔE9 mice, which were used as a transgenic mouse model of AD, results in lower levels of Aβ peptides and less accumulation of amyloid deposits [5]. Conversely, the metabolism of Aβ might be influenced by PGE₂. Therefore, PGE₂ may play a key role in the early stages of AD pathogenesis, especially in Aβ-related events.

PGES is a membrane-associated protein involved in eicosanoid and glutathione metabolism. There are three types of PGES: cPGES, mPGES-1 and mPGES-2. Cytosolic glutathione (GSH)-dependent prostaglandin (PG) E2 synthase (cPGES) is a terminal enzyme of COX-mediated PGE2 biosynthetic pathway. Functional coupling between COX-1 and cPGES was reported to be important for the synthesis of PGE2 [6]. Microsomal PGES-1 (mPGES-1) is a perinuclear protein that is markedly induced by proinflammatory stimuli, and is functionally coupled with COX-2.

Microsomal PGES-2 (mPGES-2), another PGES, is a Golgi membrane-associated protein. The proteolytic removal of its N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme [7,8]. In addition, over-expression of full-length mPGES-2 was found to be spontaneously converted to the N-terminal truncated form. This truncated version of the enzyme is similar in size to the deletion mutant mPGES-2, which lacks 87 amino acids at the N-terminal [8]. The cleavage of mPGES-2 regulates its enzymatic activities.

Beta-site APP-cleaving enzyme 1 (BACE-1), also known as beta-amyloid-converting enzyme 1, has been identified as a membrane-bound aspartic protease, chiefly located in the Golgi apparatus.

Amyloid precursor protein (APP) is first cleaved by BACE-1 and subsequently by gamma-secretase to generate beta-amyloid. Inhibitors of BACE-1 are proposed therapeutics for AD. Recent reports indicate that novel substrates are cleaved by BACE-1 [9,10,11]. Proteolytic cleavage can control the fate of enzymes, and thus BACE-1 might contribute to the regulation of enzymes.

It has been suggested that BACE-1 is one of the stress-responsive proteins. In this study, we investigated the function of BACE-1 in A β -induced inflammation, and revealed that mPGES-2 might be cleaved by and colocalized with BACE-1.

Materials and methods

Reagents. The drugs and materials used in these experiments were obtained from the following sources: human amyloid beta peptide 1-42 (Abeta 1-42) (Peptide Institute, Osaka, Japan); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) (Nacalai tesque, Kyoto, Japan); primary neuron culture materials (Invitrogen, CA, USA); 4', 6-diamidino-2-phenylindole (DAPI) and fura-2 fluorescence reagent (Dojindo, Tokyo, Japan); MTX-LDH Kit (Kyokuto, Tokyo, Japan); anti-BACE-1 (Millipore, MA, USA); anti-mPGES-1, anti-mPGES-2, anti-cPGES, and anti-COX-2 antibodies (Cayman Chemical, MI, USA); and anti-Lamin A (Abcam, MA, USA).

Neuron cultures. The use of experimental animals in this study was conducted in accordance with the ethical guidelines of the Kyoto University Animal Experimentation Committee. Primary neuron cultures were obtained from a fetal rat cerebral cortex (18 days gestation) following previously described procedures. Briefly, cells that dissociated from the cerebral cortex of fetal rats were plated out onto 48-well or 6-well tissue culture plates with a cell density of approximately 1.8×10^5 cells/cm². Cultures were incubated in neurobasal medium with 2% B27 supplement without antioxidants, 25 μ M Glutamate, and 0.5 mM L-glutamine. After 4 days in vitro, the medium was replaced by glutamate free medium (neurobasal medium supplemented 2% B27, and 0.5 mM L-glutamine). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO₂. Only mature cultures (10-11 days in vitro) were used.

Astrocyte cultures. Primary astrocyte cultures were obtained from postnatal day 1 (P1) rat cortex. Briefly, dissociated cells were seeded into 75-cm² tissue culture flasks and incubated for 20-24 days in Eagle's minimum essential medium (EMEM) supplemented with 10% FBS and 50 μ g/ml

kanamycin at 37°C in a 5% CO₂/95% air humidified incubator. Flasks were shaken at 400 rpm (10 min, 37°C) and then at 220 rpm (15 hr, 37°C). Astrocyte cultures were detached with solution minimal essential medium (SMEM) containing 0.25 µg/ml trypsin and were cultured in Dulbecco's modified Eagle's Medium (DMEM) (10% FBS and 50 µg/ml kanamycin) at 2.5×10^4 cells/cm².

Microglial culture. Microglial cells were obtained from the cortex of postnatal day 0–1 rats. For the induction of differentiation, the enriched microglial cells were cultured in DMEM supplemented with 10% FBS and 10 ng/ml macrophage colony stimulating factor (M-CSF; R&D Systems, Minneapolis, MN) for three days (days 1–3), in DMEM containing 70% FBS and M-CSF for two days (days 4 and 5), and then in serum-free DMEM in the presence of 20 ng/ml bFGF (PeproTech, London, UK) for four days (days 6–9).

Measurement of Cell Viability. Neuronal cell viability in culture was estimated by a MTT reduction assay. Briefly, treated cells (in a 48-well plate) were incubated at 37 °C for 30 minutes with media that included MTT (0.1 mg/mL in neurobasal media, 200 µL). After incubation, the MTT solution was replaced with 2-propanol (200 µL), followed by 1 min of shaking. Next, this solution was transferred to a 96-well plate, with each well containing 180 µL of solution. Absorption was measured at 570 nm by a microplate spectrophotometer (Model 680 plate reader BioRad, USA). The cell viability was expressed as the percentage of surviving cells and compared to the 100% survival observed in control cultures.

Preparation of cell extracts and western blotting. After each treatment, cells were lysed in a buffer consisting of 20 mM Tris/HCl, pH 7.0, 2 mM EGTA, 25 mM 2-glycerophosphate, 1% Triton X-100 and a protease inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). Then, the cell extracts were centrifuged at 16,200 g for 20 minutes at 4 °C. The supernatants were used as the cell extracts for

immunoblot analysis. The protein concentration of each sample was determined using a micro BCA Protein Assay kit (Piers, CA, USA). Protein samples in sodium dodecyl sulfate buffer were loaded onto sodium dodecyl sulfate-polyacrylamide gels. After electrophoresis, proteins were electrotransferred to a polyvinylidene difluoride membrane (Millipore, CA, USA). Membranes were incubated with antibodies in 20 mM Tris/HCl, pH 7.6, 135 mM NaCl, 0.1% Tween 20 containing 5% nonfat dry milk. Subsequently, membranes were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibodies. Immunoreactive bands were detected by enhanced chemiluminescence (GE Healthcare, CA, USA). Immunoblots were visualized by a ChemiDoc CCD camera detection system (Bio-Rad, CA, USA), and quantitatively analyzed by Quantity-One software (Bio-Rad, CA, USA).

Evaluation of the cleavage of mPGES-2 peptide by RP-HPLC. A synthetic 20-amino acid peptide component of mPGES-2 that included the cleavage site (HTARWHL RAQDLHERS AAQLSLSS; indicated in Figure 4) was prepared according to the sequence indicated in EMBL Nucleotide Sequence Database (Accession number AK024100). Cleavage of the peptide was analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) using a C-18, YMC column. Peptides were separated using a stepwise gradient of acetonitrile. The peptide was dissolved in DMSO and diluted with assay buffer. Subsequently, 30 μ L of peptide and 1 μ L of recombinant human BACE-1 were mixed.

Immunocytochemistry. For the immunofluorescence staining, cells were fixed with 0.1 M phosphate buffer containing 4% paraformaldehyde for 15 min. Fixed cells were rinsed with PBS three times and blocked with 5% goat serum in PBS with 0.005% saponin for 30 min and incubated overnight at 4 °C with anti-BACE-1 (1:200), anti-mPGES-2 (1:250), anti-COX-2 (1:300), or anti-Lamin A (1:500) antibodies diluted in PBS containing 1% goat serum and 0.005% saponin. The next day, the

cells were washed three times and then incubated for 1 h at room temperature with Cy3-AffiniPure goat anti-mouse IgG (1:1600) or Cy2-AffiniPure goat anti-mouse IgG (1:1600). Cultures were washed with PBS, treated with FluorSave Reagent (Calbiochem, Germany), covered, and examined under an Olympus photomicroscope equipped for epifluorescence. Digital images were obtained with a CCD camera.

PGE2 and Abeta enzyme-linked immunosorbent assay (ELISA). After cultures were treated in neurobasal medium containing 2% B27 supplement for 24 h at 37 °C, debris was removed by centrifugation, and the media were then applied to ELISA plates. The culture media were analyzed for prostaglandin production using a monoclonal PGE2 enzyme-linked immunosorbent assay (ELISA) (Cayman Chemicals, MI, USA), or for Abeta 1-40 or Abeta 1-42 using Human/Rat Abeta 1-40 or Abeta 1-42 ELISA Kit (Wako Pure Chemical, Tokyo, Japan), following the manufacturer's instructions.

Statistical Analysis. Statistically significant differences between groups were determined by an analysis of variance followed by a Dunnett or Newman-Keuls post-hoc analysis. The level of statistical significance was taken at $p < 0.05$.

Results

Abeta induces the production of PGE2

To investigate Abeta-induced inflammation, we measured extracellular PGE2 levels induced by Abeta, using an ELISA. Administration of Abeta 1-42 for 48 h significantly increased PGE2 levels in the media of neuronal culture (Fig. 1A). Simultaneous administration of BACE-1 inhibitor IV (200 nM) significantly suppressed Abeta 1-42-induced PGE2 up-regulation (5 μ M, 48 h). These results suggest BACE-1 is involved in the production of PGE2 induced by Abeta. In contrast, exposure of neuronal cells to lipopolysaccharide (LPS) also significantly increased PGE2. Secreted levels of PGE2 were more prominent than PGE2 levels resulting from Abeta stimulation, and BACE-1 inhibitor IV did not suppress LPS-induced PGE2 up-regulation (Fig. S1).

Previously, we demonstrated that exposure of Abeta 1-42 to neuronal cultures increased BACE-1 protein levels [12]. As the Abeta peptide might form a cross-linked complex directly with COX-2, we investigated the effect of indomethacin, a COX inhibitor, upon Abeta-induced BACE-1 up-regulation (Fig. 1B). Administration of Abeta 1-42 for 48 h significantly increased BACE-1 expression levels. Simultaneous administration of indomethacin (10 μ M) significantly suppressed Abeta 1-42-induced BACE-1 up-regulation (5 μ M, 48 h) in a concentration dependent manner. Ibuprofen also attenuated the BACE-1 up-regulation induced by Abeta (data not shown). These results suggest COX activation may be involved in the increase of BACE-1 levels.

Abeta enhances the protein levels of BACE-1, COX-2 and PGE2 synthase

We next investigated the cell type responsible for the production of PGE2. cPGES, mPGES-1, and mPGES-2 are responsible for the synthesis of PGE2. Therefore, the protein levels of these enzymes were analyzed by western blotting (Fig. 2A).

Exposure to Abeta increased the protein level of mPGES-2 significantly in pure neuron and astrocytic cultures. On the other hand, Abeta did not affect cPGES levels in any cell culture. In all cultures, mPGES-1 was not detected. These data suggest that mPGES-2 may be an inducible enzyme that is responsive to Abeta.

COX-2 activity can also be induced by various stimuli or stress. It has been reported that expression of COX correlates with levels of Abeta. Therefore, we evaluated the expression level of COX-2 using pure neuronal, astrocyte, and microglia cultured cells. As shown in Figure 2A, COX-2 levels were enhanced by Abeta treatment in all cell cultures.

Although BACE-1 was not detectable in the microglia culture, BACE-1 expression was determined to be enhanced in the pure neuronal culture and in the astrocyte culture.

N-terminal 20-residue peptide of mPGES-2 was cleaved by BACE-1

It has been shown that 87-residues of the N-terminus of mPGES-2 can be cleaved in the cytoplasm, resulting in an active form of mPEGS-2. This fragmentation leads to the release of mPGES-2 from the Golgi membrane and allows mPGES-2 to migrate to other intracellular organs. In order to clarify the involvement of the cleavage of the N-terminal sequence by BACE-1, we prepared a 20-residue peptide including the 87th amino acid sequence of mPGES-2 (Fig. 2B). After the in vitro reaction of this synthetic peptide (20AA) with recombinant BACE-1, the peptide was separated by reverse HPLC using a C18 column.

HPLC analysis revealed a single peak when the 20AA peptide was administered alone. Following the reaction of 20AA with BACE-1, three peaks were found. Two of the peaks might represent the cleaved peptides, while the other is the original peak. These results suggest that BACE-1 can cleave mPGES-2.

PGE2-induced neuronal death

Simultaneous treatment with BACE-1 inhibitor IV, which reduced Abeta-induced neuronal death, also reduced Abeta-induced PGE2 production. We therefore investigated whether PGE2 is toxic to neurons using MTT assay. As a result, administration of synthetic PGE2 induced neuronal death in a concentration dependent manner (Fig. 3A). From these data, we concluded that Abeta administration leads to the augmentation of PGE2 mediated via BACE-1, which in turn induces, at least in part, neuronal death.

Extracellular Abeta secretion was increased by PGE2-related inflammation

Previously, it was indicated that PGE2 caused Abeta secretion [13]. We also confirmed that Abeta 1-40 or 1-42 levels were augmented by PGE2 treatment. Synthetic PGE2 treatment significantly increased the Abeta 1-40 and 1-42 levels in a concentration-dependent manner (Fig. 3B, C).

Colocalization of mPGES-2 and BACE-1 or COX-2 was induced by Abeta

To clarify whether mPGES-2 colocalizes with BACE-1 or COX-2, mixed culture cells were immunostained and analyzed by photomicrographs. mPGES-2 colocalized with BACE-1 in cells after exposure to Abeta (Fig. 4A).

While COX-2 did not colocalize with mPGES-2 in a resting state (Fig. 4B), administration of Abeta led to the colocalization of mPGES-2 and COX-2. Simultaneous administration of the BACE-1 inhibitor attenuated the Abeta-induced colocalization of mPGES-2 and COX-2. Exposure to Abeta induced the assemblies of mPGES-2, BACE-1 and COX-2 in the perinuclear region (Fig. 4B). COX-2 is mainly located in the nuclear membrane. Lamin-A, a nuclear membrane marker, colocalized with mPGES-2 after exposure to Abeta (Fig. 4C). Simultaneous administration of the BACE-1 inhibitor attenuated this phenomenon.

These data suggest that Abeta induces the association of BACE-1 and mPGES-2, which causes the migration of mPGES-2 to the perinuclear region where COX-2 is located.

Discussion

BACE-1 is an aspartic protease, and cleavage of APP has been investigated for the elucidation of AD pathogenesis. In addition to APP, novel BACE-1 substrates have recently been identified [9,10,11]. BACE-1 might be activated by various stress stimuli, and it might therefore cleave stress-reactive peptides.

mPGES-2 is synthesized in the Golgi membrane, and it then undergoes a proteolytic event where its N-terminal hydrophobic domain is removed. This truncated enzyme is subsequently released into the cytoplasm. Abeta stimulation induced the translocation of mPGE2 to perinuclear region where BACE-1 also assembles. Therefore, it is possible that BACE-1 cleaves mPGES-2 in the perinuclear region. Further supporting this hypothesis is the fact that mPGES-2 possesses one hydrophobic region, and it is therefore possible that its enzyme structure is similar to that of APP, another known BACE-1 substrate.

The 20-amino acid synthetic mPGES-2 peptide was determined by EMBL Nucleotide Sequence Database (Accession number AK024100) to contain the N-terminal 87th amino acid sequence of PGE2. The full-length protein of mPGES-2 is spontaneously converted to an N-terminal truncated form, which is similar in size to the reported deletion mutant of mPGES-2 that lacks the N-terminal 87th amino acid sequence [8]. We hypothesized that since either mature or truncated mPGES-2 has been reported to exhibit bioactivity, that cleavage product of PGE2 mediated by BACE-1 would be bioactive, we therefore prepared this peptide.

Full length mPGES-2 is associated with the Golgi membrane, and it is dispersed in the cytoplasm after removal of the N-terminal region [8]. However, our data suggest that Abeta-stimulated cells exhibit assemblies of mPGES-2 and BACE-1 around the nucleus. IL-1beta-treated fibroblasts show overlapping expression of COX-2 and mPGES-2 primarily in the perinuclear region [14]. mPGES-2

is constitutively synthesized and is not regulated by IL-1 β . Some stimuli might cause the translocation of mPGES-2 without up-regulation in protein synthesis.

It has been reported that both COX-1 and COX-2 mediate mPGES-2-promoted PGE₂ production, with COX-2 being slightly preferred [8]. In our data, A β induced the translocation of COX-2 from the nucleus to the perinuclear region, where mPGES-2 was also found. Treatment with a BACE-inhibitor attenuated this phenomenon, indicating the involvement of BACE-1 in this process. BACE-1-induced cleavage of mPGES-2 would activate the enzyme promoting its translocation to the perinuclear region where it could couple with COX-2 and contribute to the production of PGE₂. In addition to the increases in the processing and translocation of mPGES-2, its protein levels were also augmented. The induction of PGE synthase mRNA levels by treatment of cells with A β has previously been reported [15]. However, the precise mechanism is not known, and it is not clear why mPGES-2 mRNA was up-regulated.

Previously we have reported that A β treatment induces increases in BACE-1 protein levels [12]. This change may be due to increased BACE-1 transcription by A β [16]. BACE-1 may in turn cleave APP to produce more A β . In this study, we demonstrated that A β treatment leads to the production of PGE₂, which up-regulates A β .

It has been reported that PGE₂ stimulates the production of A β [13]. Specifically, PGE₂ induces internalization of presenilin 1 (PS1), followed by the activation of gamma-secretase [17]. A β simultaneously up-regulates BACE-1, which causes ectodomain shedding of APP. APP is subsequently cleaved to produce A β , which propagates the vicious cycle of AD pathogenesis. Our data demonstrated that PGE₂ treatment induced neurotoxicity. A β -induced PGE₂ levels or PGE₂-induced A β levels were not sufficient in quantity to result in neurotoxicity. Previously, we have shown that A β -induced neurotoxicity is attenuated by treatment with BACE-1 inhibitors [12]. Neuroinflammation induced by PGE₂ may aggravate AD pathogenesis in brains through this positive feedback cycle.

In conclusion, Abeta causes BACE-1 up-regulation, which cleaves mPGES-2, which is subsequently translocated to the perinuclear region where it synthesizes PGE2 collaboratively with COX-2. As a result, increased BACE-1 levels and neuroinflammation would lead to the up-regulation of Abeta.

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Legends to figures

Fig. 1. Abeta induced production of PGE2 and enhanced the protein level of BACE-1 via

COX-2. (A) Neuronal cultures were exposed to Abeta 1-42 for 48 h, and media were analyzed using a monoclonal PGE2 enzyme-linked immunosorbent assay (ELISA). Abeta treatment significantly increased PGE2 levels in a concentration-dependent manner. Simultaneous administration of BACE-1 inhibitor IV (200 nM) significantly suppressed Abeta 1-42 (5 μ M, 48 h)-induced PGE2 up-regulation. Data are expressed as Mean \pm SEM, n = 4. * p < 0.05, ** p < 0.01, compared with control (CTL; vehicle alone), $^{##}p$ < 0.01, compared with Abeta 1-42 5 μ M. **(B)** Administration of Abeta 1-42 for 48 h significantly increased BACE-1 expression levels. Simultaneous administration of indomethacin (10 μ M) significantly suppressed Abeta 1-42 (5 μ M, 48 h)-induced BACE-1 up-regulation.

Fig. 2. Abeta enhanced the protein levels of PGE2 synthase, and BACE-1 was involved in the

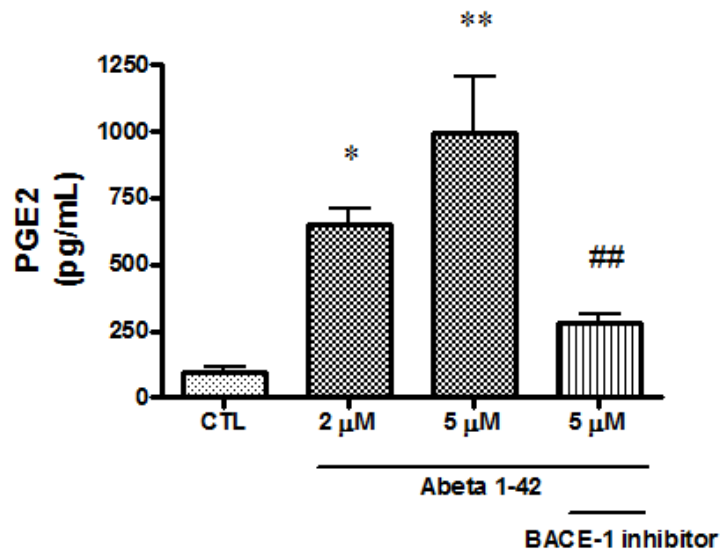
activation of mPGES-2. (A) After 48 h-exposure to Abeta 1-42 (5 μ M), cells were lysed and prepared for the immunoblot analysis. Representative data of BACE-1, COX-2 and PGE2 synthase levels. BACE-1 and mPGES-2 levels were increased by Abeta treatment in neuronal and astrocytic cell cultures. In microglial cells BACE-1 was not detected, and mPGES-2 levels were constant. COX-2 protein levels were increased in neuronal, astrocytic and microglial cell cultures. Levels of cPGES were not changed by Abeta treatment. mPGES-1 was not detected in our cultures. P.C.; positive control prepared from the recombinant protein. **(B)** Quantitative analysis of mPGES-2 protein levels. ** p < 0.01, compared with control (vehicle alone; CTL), N.S., not significant. **(C, D)** N-terminal 20-residue peptide of mPGES-2 was cleaved by BACE-1. **(C)** Schematic structures of mPGES-2 and selected 20 amino acids. **(D)** Cleavage of the peptide was analyzed by reverse-phase

high-performance liquid chromatography (RP-HPLC) using a C-18, YMC column. The 20-residue peptide was indicated as a single peak (top chromatogram), which was reduced by recombinant human BACE-1 (middle chromatogram). The bottom chromatogram represents the overlay of the 20-residue peptide chromatogram and the BACE-1 reduced peptide chromatogram.

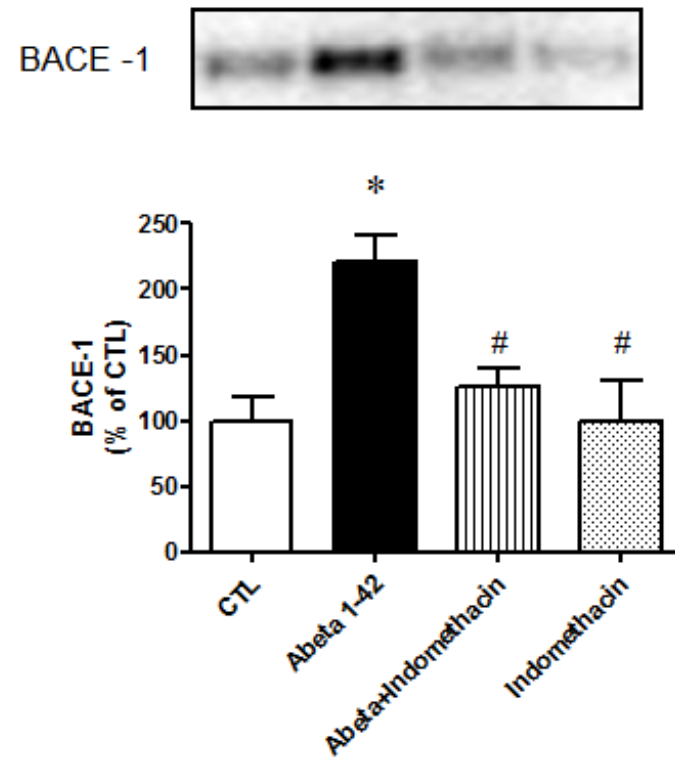
Fig. 3. PGE2 induced neuronal death and extracellular Abeta secretion. (A) Neuronal cultures were exposed to PGE2 for 48 h. Viabilities were evaluated by MTT assay. Synthetic PGE2 induced neuronal death in a concentration-dependent manner. Data are expressed as Mean \pm SEM, $n = 4$ ** $p < 0.01$, compared with control (vehicle alone; CTL). (B), (C) Abeta 1-40 or Abeta 1-42 in the media was quantified using ELISA assays. Synthetic PGE2 treatment significantly increased the Abeta 1-40 and 1-42 levels in a concentration dependent manner. Data are expressed as Mean \pm SEM, 5 of control. $N = 4$. * $p < 0.05$, ** $p < 0.01$, compared with control (vehicle alone; CTL)

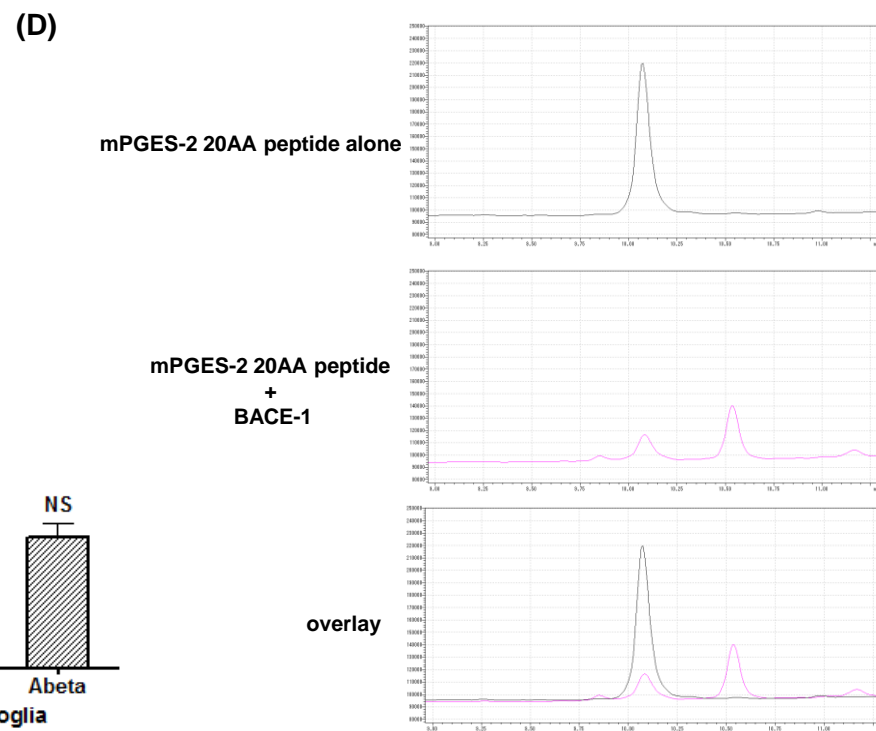
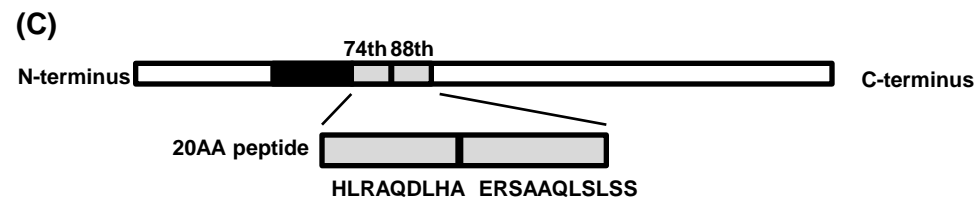
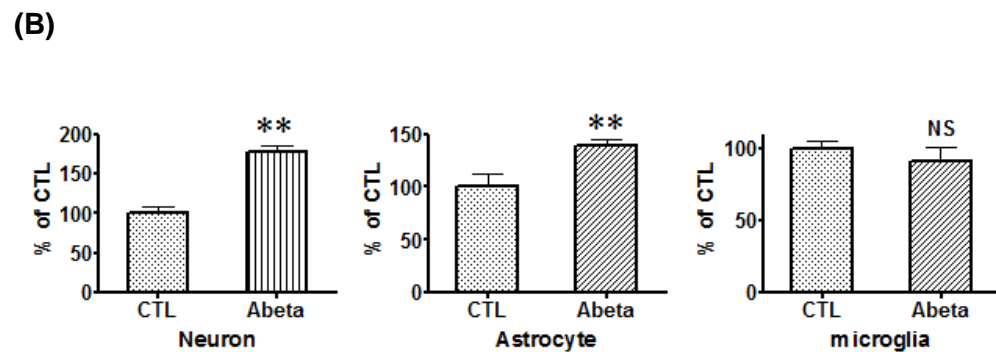
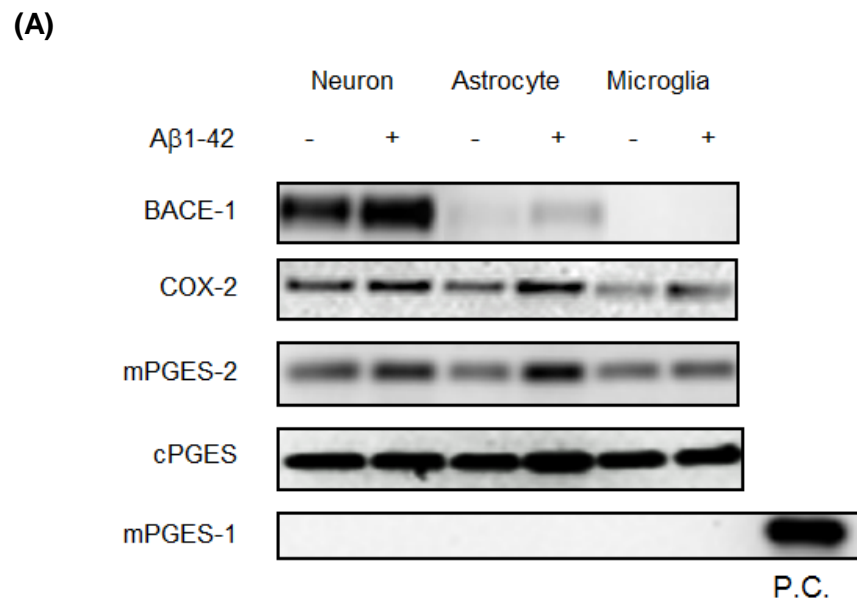
Fig. 4. Abeta induced colocalization of BACE-1, mPGES-2 and COX-2. (A) Exposure to Abeta led to the colocalization of mPGES-2 and BACE-1. (B) Exposure to Abeta led to the colocalization of mPGES-2 and COX-2. Simultaneous administration of BACE-1 inhibitor (BACEI) attenuated the Abeta-induced colocalization of mPGES-2 and COX-2. (C) Lamin-A, a nuclear membrane marker, was colocalized with mPGES-2 after exposure to Abeta. Simultaneous administration of BACE-1 inhibitor (BACEI) attenuated this phenomenon.

(A)

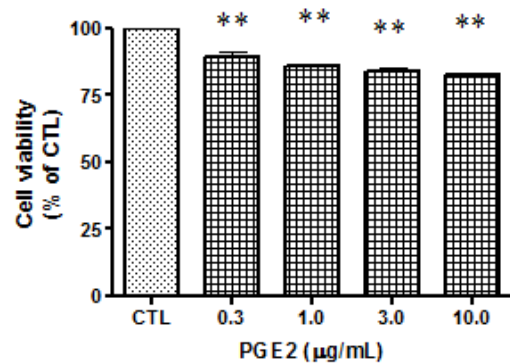


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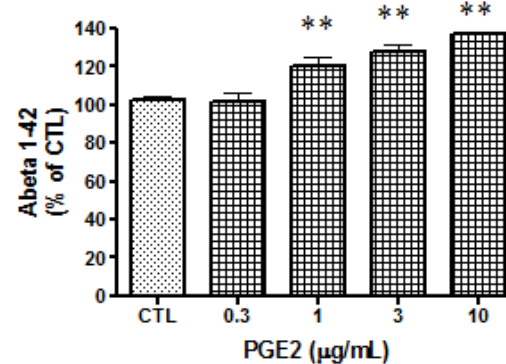




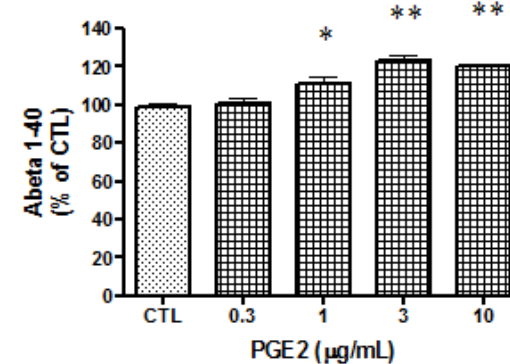
(A)



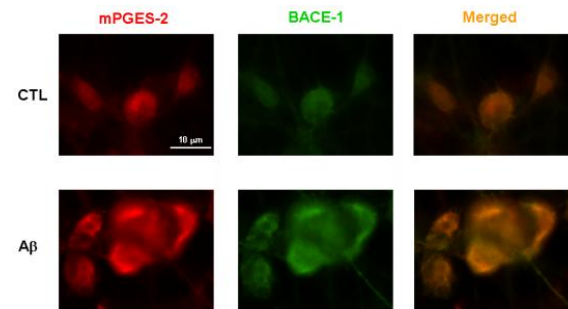
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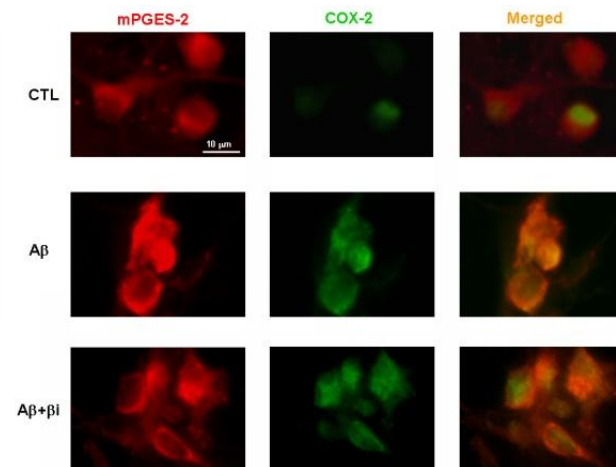
(C)



(A)



(B)



(C)

